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Expression of Potential Target Antigens for Immunotherapy on Primary and Metastatic Prostate Cancers¹

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ABSTRACT

Defining the expression of tumor-associated antigens on primary and metastatic prostate cancer is the crucial first step in selecting appropriate targets for immune attack. In this study, the distribution of the tumor-associated antigens GM2, Tn, sTn, Thompson-Friedenreich antigen (TF), Globo H, Le^x, MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC7, carcinoembryonic antigen, β chain of human chorionic gonadotropin (hCG β), HER2/neu, PSMA, and KSA on primary and metastatic prostate cancer and 16 types of normal tissues was compared by immunohistochemistry, using a panel of well-characterized monoclonal antibodies. Our results show that GM2, KSA, and MUC2 were strongly expressed on 8 or 9 of 9 metastatic prostate cancer biopsy specimens and, with PSMA, hCG β , TF, Tn, and sTn, on 8 or more of 11 primary prostate cancer specimens. Tn, MUC1, and PSMA were expressed on 4-6 of 9 metastatic specimens. The remaining antigens were expressed on no more than three of nine metastatic specimens. Normal tissues were also tested with all antibodies. With regard to the eight antigens most widely expressed on prostate cancers, PSMA was not expressed significantly on any of the normal tissues except prostate epithelium. Tn, sTn, hCG β , and MUC2 were detected on up to 3 of 10 types of normal epithelia. GM2, TF, MUC1, and KSA were more broadly distributed on normal epithelia, all primarily at the secretory borders. STn, KSA, and hCG β were also detected in the testis, and GM2 was expressed on gray matter of brain. From the 30 antigens that we have screened, this study provides the basis for selecting GM2, TF, Tn, sTn, hCG β , MUC1, MUC2, KSA, and PSMA as target antigens for specific immunotherapy of prostate cancer.

INTRODUCTION

The progression of prostate cancer from the hormone-naïve primary to increasingly androgen-independent metastatic lesions is associated with a number of molecular and genetic changes. These changes can affect the expression of specific antigens on the cell surface. Defining the expression of tumor-associated antigens on prostate cancers of different stages is the crucial first step in selecting targets for specific immunotherapy. We have previously used a panel of murine mAbs³ to determine the expression of 18 different tumor-associated carbohydrate antigens on a broad range of malignancies, including five hormone-naïve primary prostate cancer specimens, by immunohistochemistry (1, 2). GM2, Tn, sTn, and TF were expressed in four or five of five primary prostate cancers, Le^x was expressed on three of five and Globo H was expressed on two of five primary prostate cancers. Twelve other antigens were expressed on one or none of the five specimens. Metastatic prostate cancer specimens were not tested.

In this report, we have (a) increased the number of primary hormone-naïve cancers evaluated; (b) extended the work to include metastatic lesions; (c) evaluated the expression on this expanded tumor panel of the six carbohydrate antigens expressed on more than one of five primary cancers; and (d) evaluated the expression of 12 protein tumor antigens: MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC7, hCG β , HER2/neu, PSMA, KSA, and CEA. This study provides a comprehensive basis for selecting cell surface target antigens for specific immunotherapy of prostate cancers with mAbs or vaccines.

MATERIALS AND METHODS

Tissue Samples. Frozen specimens embedded in Tissue-Tek OCT compound (Diagnostic Division, Elkhart, IN) were provided with pathological reports by the Tissue Procurement Service of MSKCC, with the exception of four frozen specimens of metastatic prostate cancer kindly provided by Dr. G. Steven Bova (Pelican Laboratory, Johns Hopkins University). Cryostat sections were cut at 5 μ m, dried in air, and fixed with neutral buffered 10% formalin solution (Sigma Chemical Co., St. Louis, MO) for 10 min before H&E or immune staining.

mAb and Immunohistochemistry. The murine mAbs and the antigens they recognize are summarized in Table 1. mAb 696 was provided by Nobuo Hanai (Kyowa Hakko Kogyo Co., Tokyo, Japan); 1E3 by A. K. Singhal (The Biomembrane Institute, Seattle, WA); B72.3 and CC49 by J. Schlom (National

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³ The abbreviations used are: mAb, monoclonal antibody; ABC, avidin-biotin complex; CEA, carcinoembryonic antigen; PSMA, prostate-specific membrane antigen; TF, Thompson-Friedenreich antigen; hCG β , β chain of human chorionic gonadotropin; MSKCC, Memorial Sloan-Kettering Cancer Center.

Table 1 Mouse mAbs studied

mAb	Immunoglobulin Class	Antigen	Antigen structure	Ref.
696	IgM	GM2	GalNAc β 1 \rightarrow 4(NeuAc α 2 \rightarrow 3) Gal β 1 \rightarrow 4Glc β 1 \rightarrow 1Cer	3
49H.8	IgM	TF	Gal β 1 \rightarrow 3GalNAc α -O-serine/threonine	4
1E3	IgG2b	Tn	GalNAc α -O-serine/threonine	Unpublished ^a
B72.3	IgG1	sTn	NeuAc α 2 \rightarrow 6GalNAc α -O-serine/threonine	5
MBr1	IgM	Globo H	Fuca1 \rightarrow 2Gal β 1 \rightarrow 3GalNAc β 1 \rightarrow 3Gal α 1 \rightarrow 4Gal β 1 \rightarrow 4Glc β 1 \rightarrow 1Cer	6
3S193	IgG3	Le ^x	Fuca1 \rightarrow 2Gal β 1 \rightarrow 4(Fuca1 \rightarrow 3)GlcNAc β 1 \rightarrow 3Gal	7
HMFG-2	IgG1	MUC1	VTSAPDTRPAGSTAPPAHG repeating	8
LDQ10	IgM	MUC2	PTTTPISTTTTVPPTPTPTGTQT repeating	9
M3.2	IgG2a	MUC3	HSTPSFTSSITTTETTS repeating	10
MUC4.275	IgG	MUC4	TSSASTGHATPLPVTD repeating	10, 11
CLH2	IgG1	MUC5AC	TTSTTSAP repeating (interrupted)	12
PANH2	IgG1	MUC5B	No peptide repeats	13, 14
PANH3	IgG1	MUC7	TTAAPPTPSATTAPPSSSAPPE repeating	13, 14
NCL-CEA	IgG1	CEA	Glycoprotein (M_r 180,000)	Vector Co.
Cyt351	IgG	PSMA	Protein (M_r 100,000)	15-17
GA733-2	IgG2a	KSA(EGP-2)	Glycoprotein (M_r 40,000)	18
FB12	IgG1	hCG β	145-amino acid glycoprotein	19
NCL-CBE1	IgG2a	HER2/neu	Protein (M_r 185,000)	20

^a A. Singhal and S. Hakomori, unpublished data.

Cancer Institute, Bethesda, MD); 49H.8 by R. Koganty (Biomira Inc., Edmonton, Alberta, Canada); MBr1 by M. I. Colnaghi (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy); 3S193 by L. J. Old (MSKCC); HMFG-2 by J. Taylor-Papadimitriou (Imperial Cancer Research Fund, London, United Kingdom); LDQ10 by F. X. Real (Institut Municipal d'Investigacio Medica, Barcelona, Spain); M3.2 and MUC4.275 by V. Apostolopoulos (Austin Research Institute, Heidelberg, Victoria, Australia); CLH2, PANH2, and PANH3 by H. Clausen (University of Copenhagen, Copenhagen, Denmark); Cyt351 by W. Heston (MSKCC); FB12 by D. Bellet (Institut Gustave-Roussy, Villejuif, France); and GA733-2 by D. Herlyn (The Wistar Institute, Philadelphia, PA). mAbs NCL-CEA and NCL-CBE1 were purchased from Vector Laboratories, Inc. (Burlingame, CA).

The ABC immunoperoxidase method was performed as described previously (21). Briefly, the sections were quenched with 0.1% H₂O₂ in PBS for 15 min, blocked with avidin and biotin reagents (Vector Laboratories, Inc.) for 10 min each, incubated in 10% serum of horse or goat from which the second antibody was raised, and incubated with various mAbs for 1 h at optimal concentration. The optimal mAb concentration was selected based on strong reactivity against the known positive target cells and little or no background against stroma. The concentrations of mAbs used were as follows: FB12 at 0.5 μ g/ml; 696 at 0.8 μ g/ml; B72.3, CC49, Cyt351, and GA733-2 at 2 μ g/ml; 49H.8 at 5 μ g/ml; MBr1 at 1 μ g/ml; 3S193 at 1.5 μ g/ml; HMFG-2, M3.2, MUC4.275, CLH2, PANH2, PANH3, and 1E3 (supernatants) at between 1:3 and 1:6; LDQ10 (ascites) and NCL-CBE1 at 1:15; and NCL-CEA at 1:50. The sections were subsequently incubated with 1:600 biotinylated horse antimouse IgG or 1:300 goat antimouse IgM antibodies (Vector Laboratories, Inc.) for 40 min and then incubated in 1:50 ABC reagent (Vector Laboratories, Inc.) for 30 min. Reactions were developed with 0.02% H₂O₂ and 0.1% diaminobenzidine tetrahydrochloride (Sigma) for 2-5 min. Slides were then counterstained with Harris modified hematoxylin (Fisher

Scientific, Fair Lawn, NJ) for 1-3 min. The immunoreactivities were graded based on the percentage of positive cells and staining intensity above that seen on the negative control: 1+ (weak), 2+ (moderate), 3+ (strong), and 4+ (very strong). Staining intensities of 2+ or stronger were considered positive (see Table 2 and Fig. 1). Known positive and negative control slides were used in each experiment. Results with the several IgM, IgG3, and IgG2 mAbs included in the panel of antibodies tested ruled out nonspecific adherence of particular subclasses of antibodies.

An indirect immunoperoxidase assay was performed on normal liver, kidney, and stomach samples. These tissues reacted strongly with ABC reagent directly, producing high background staining. Briefly, the sections were quenched with 0.1% H₂O₂ in PBS for 15 min, blocked with 10% serum, and incubated with mAbs for 1 h at the optimal concentration. The sections were incubated with 1:100 rabbit antimouse immunoglobulin labeled with peroxidase (DAKO Co., Carpinteria, CA) for 1 h and developed as described for the ABC method.

RESULTS

Expression of Tumor-associated Antigens on Prostate Cancer. Using 50% or more of tumor cells positive per tissue section as a cutoff, GM2, KSA, MUC1, MUC2, and PSMA were expressed on five or more of nine metastatic prostate cancer specimens, and using 20% of positive cells as cutoff, GM2, KSA, MUC2, and Tn were expressed in eight or more of nine specimens (see examples of staining in Fig. 1 and the results summarized in Table 2). TF, sTn, Globo H, hCG β , and Le^x were expressed (using the 50% cutoff) on two or three of nine metastatic specimens, and CEA, HER2/neu, MUC3, MUC4, MUC5AC, MUC5B, and MUC7 were all expressed in one or none of the specimens. Primary prostate cancers expressed GM2, KSA, TF, Tn, sTn, MUC2, hCG β , and PSMA in 8 of 11 or more specimens, whereas CEA, Le^x and Globo H were expressed in 2-7 of 11. MUC3, MUC4, MUC5AC,

Table 2 Proportion of normal and tumor specimens with 50% (20%) or more of cells positive by immunohistochemistry^a

Tissue	GM2 (696)	TF (49H.8)	Tn (IE3)	sTn (B72.3)	sTn (CC49)	Globo-H (MBR1)	Le ^y (3S193)	Antigen (mAb)										HER2/ NCL- bCGB (PB12) CBE1)
								MUC1 (HMFG-2)	MUC2 (LDQ10)	MUC3 (M3.2)	MUC4 (M4.275)	MUC5AC (CLH2)	MUC5B (PANH2)	MUC7 (PANH3)	CEA (NCL- CEA)	PSMA (Cy351)	KSA (GA733-2)	
Metastatic prostate cancer	8/9 (8/9)	2/9 (5/9)	4/9 (8/9)	3/9 (4/9)	3/9 (4/9)	2/9 (2/9)	3/9 (3/9)	5/9 (5/9)	8/9 (9/9)	0/9 (1/9)	0/9 (0/9)	1/9 (1/9)	0/9 (0/9)	0/9 (2/9)	6/9 (6/9)	9/9 (2/5)	1/5 (1/5)	
Primary prostate cancer	11/11 (10/11)	10/11 (10/11)	10/11 (10/11)	6/11 (9/11)	9/11 (10/11)	2/11 (6/11)	4/11 (6/11)	0/11 (3/11)	8/11 (8/11)	0/11 (0/11)	0/11 (0/11)	1/11 (1/11)	0/11 (1/11)	7/11 (9/11)	8/11 (10/11)	11/11 (10/11)	0/11 (0/11)	
Prostate glandular epithelia	6/6	0/2	0/6 (2/6)	0/6 (2/6)	0/6 (1/6)	3/6 (4/6)	2/6 (3/6)	1/6 (2/6)	2/6 (5/6)	0/6 (2/6)	0/6 (1/6)	1/6 (1/6)	0/6 (0/6)	4/6 (5/6)	3/6 (5/6)	6/6 (3/6)	0/6 (0/6)	

^a All of the tumor tissues were stained by ABC immunoperoxidase methods.

MUC5B, MUC7, and HER2/neu were detected on one or none of the specimens tested. Interestingly, MUC1 was present on five of nine metastatic prostate cancers but was not strongly expressed on any primary cancers.

The correlation between staining intensity and percentage of positive tumor cells in the nine metastatic specimens was determined (see Table 4). GM2, MUC2, and KSA stained 60-95% of tumor cells in at least eight of nine metastatic prostate cancer specimens, generally with strong or very strong staining. These antigens were also expressed on at least 90% of most primary prostate cancers, with a staining intensity of 4+ (data not shown). Six of nine metastatic specimens also expressed PSMA with 3+ or greater intensity. Samples of the staining against these antigens on metastatic prostate cancer specimens and the corresponding percentage of positive cells and staining intensity are demonstrated in Fig. 1. The lack of background staining on normal stroma or adjacent normal tissues was a universal finding with these antibodies used at these concentrations.

Expression of Tumor-associated Antigens on Normal Tissues. In addition to normal prostate tissue, many other normal tissues were tested. Examples are shown in Fig. 2 and the results are summarized in Table 3. GM2 was distributed on gray matter of brain and the epithelia of all tested organs except liver. Tn was expressed on epithelia of stomach and ovary. sTn, defined by mAb B72.3 and CC49, was expressed on Leydig cells of testis and on ovarian and gastric epithelia. mAb CC49 also reacted with epithelia of colon and pancreas. TF was detected on 5 and Globo H and Le^x were detected on 7 epithelia of the 10 tested. MUC1 was weakly distributed on the epithelia of all of the tested organs except liver. MUC2 was observed on the epithelia of prostate, colon, and pancreas. MUC3 was only detected on epithelia of pancreas. MUC4 was expressed on epithelia of colon and prostate (weakly). MUC5AC was strongly expressed in stomach epithelium. MUC7 and HER2/neu were not expressed on any normal tissues, and MUC5B was only detected on normal colon epithelium and, weakly, in the testis. hCG β was detected in epithelia of prostate, stomach, and pancreas, and weakly in colon and lung, and it was detected in the testis. PSMA was only detected on prostate epithelia and, weakly, on skeletal muscle cells. KSA was strongly expressed on the epithelia of all of the tested organs except stomach and liver, and it was moderately expressed on seminiferous tubules of testis. The pattern of expression of each of these antigens on normal epithelia was mainly luminal, with polarity evident. Antigen expression was primarily at the luminal surface of positive cells.

DISCUSSION

Immunohistochemistry is notoriously inconsistent for quantitating antigen expression, even when performed by an experienced investigator, because the results obtained are dependent on precise mAb specificity, affinity, and concentration. We have attempted to limit these variables by selecting well-studied mAbs and a consistent method for selecting the optimal concentration of each mAb. In addition, we have not been able to determine by immunohistochemistry whether the antigens are being expressed at the cell surface or intracellularly. Although all of

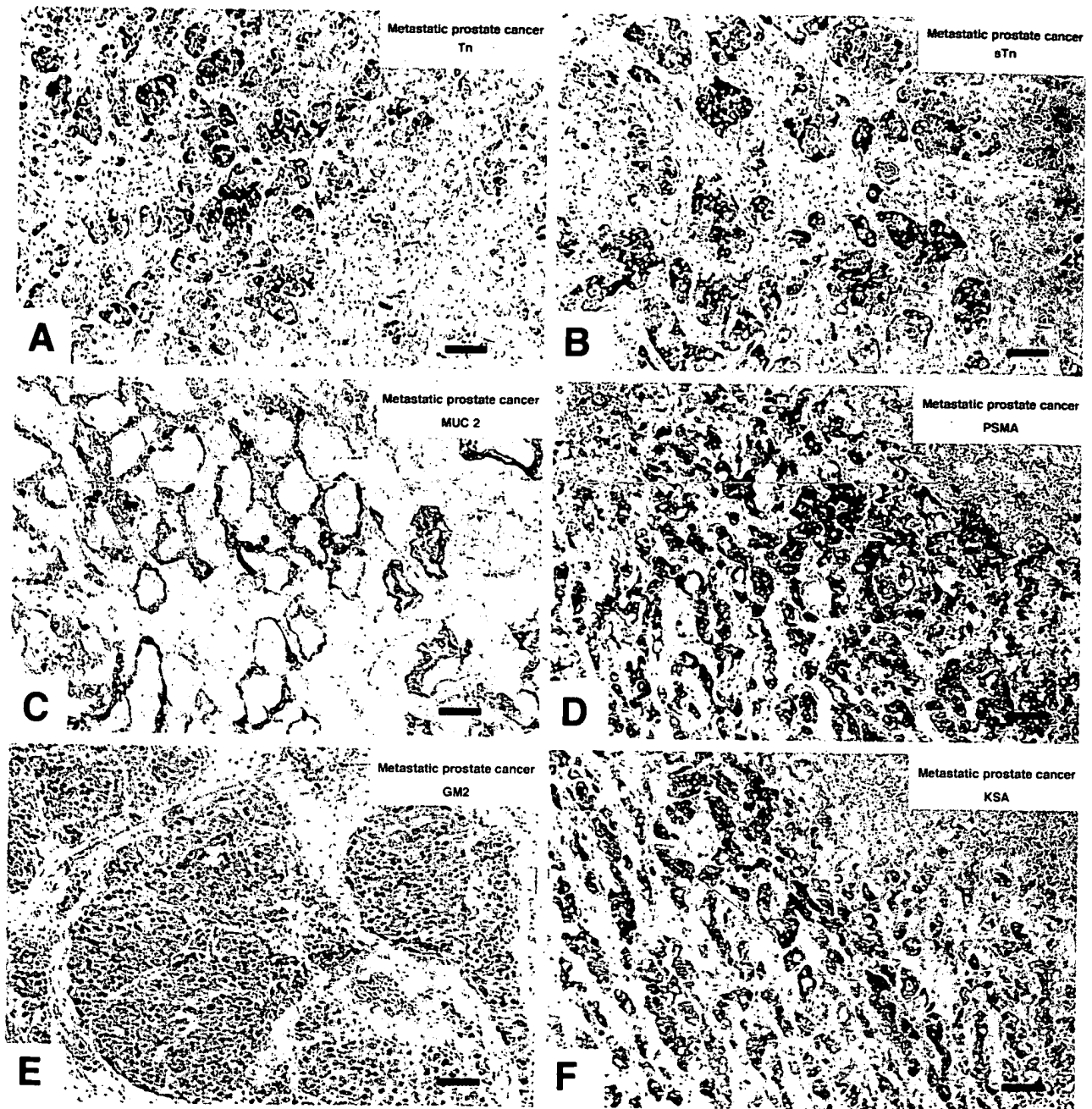


Fig. 1 Expression of tumor-associated antigens on metastatic prostate cancer. Strong immune staining was detected on metastatic prostate cancer in bone marrow with mAb 1E3 against Tn (A: 80% 3-4+) and mAb B72.3 against sTn (B: 80% 3-4+); in lung with mAb LDQ10 against MUC2 (C: 80% 4+); and in lymph node with mAb Cyt351 against PSMA (D: 95% 4+), mAb 696 against GM2 (E: 90% 4+), and mAb GA733-2 against KSA (F: 95% 3+) the absence of staining on stroma. Scale bar, 100 µg.

the antigens selected (with the exception of hCG β and mucins MUC2-7) are known to be generally expressed at the cell surface, our studies cannot confirm this in these particular cancers and normal tissues. With these provisos, this study identified nine prostate cancer cell surface antigens that are candidate targets for immune attack. The criteria for selecting target antigens for study in immunotherapy trials depends on the frequency of staining in specimens from different patients'

primary and metastatic tumors, as well as the percentage of cells positive in each specimen and the staining intensity. It also includes the specificity of this staining, including the distribution pattern of the antigen on various normal tissues. GM2, KSA, and MUC2 were expressed strongly on at least 8 of 9 metastatic prostate cancer specimens and 8 of 11 primary cancers. Tn, MUC1, and PSMA were strongly expressed in between 4-6 of the 9 metastatic specimens, and TF, Tn, sTn, hCG β , and

Table 3 Antigen expression on normal tissues defined by immunohistoLOGY^a

Normal tissue (No.) ^a	Antigen (mAb)														HER/ NCL- CBE1	
	GM2 (696) (49H.8)	TF (1E3)	Tn (B72.3) (CC49)	sTn (B72.3) (CC49)	Globo H (MB1)	Le ^x (3S193) (HMFG-2) (LDQ10)	MUC1 (M3.2) (M4.275)	MUC2 (M3.2) (M4.275)	MUC3 (M3.2) (M4.275)	MUC4 (CLH2)	MUC5AC (PANH2)	MUC5B (PANH2)	MUC7 (PANH3)	CEA (NCL-CEA)	PSMA (Cy351) (GA733-2)	KSA (hCGβ (FB12) CBE1)
Brain (3)																
Gray matter	2+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
White matter	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spleen (2)																
White pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymph node (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Striated muscle (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Smooth muscle (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Epithelia																
Lung (2)	3+	2+	-	-	3+	2+	2+	-	-	-	-	-	-	1+	-	1+
Breast (2)	3+	2+	-	-	3+	2+	1+	-	-	-	-	-	-	2+	-	2+
Prostate (6)	4+	2+	+/-	+/-	2+	3+	+/-	2+	2+	+/-	-	-	-	3+	3+	4+
Colon (2)	4+	2+	-	2+	-	-	2+	3+	-	3+	-	3+	-	3+	4+	1+
Stomach (2)	3+	2+	2+	2+(1/2)	3+	3+(1/2)	1+	-	-	4+	-	-	1+	-	-	-
Pancreas (2)	3+	2+	2+	2+(1/2)	4+	3+	2+	2+	-	-	-	-	-	1+	-	-
Uterus (2)	3+	-	-	-	4+	2+	1+	-	-	-	-	-	-	-	-	-
Ovary (2)	3+	2+	2+(1/2)	3+(1/2)	4+	3+(1/2)	1+	-	-	-	-	-	-	-	-	-
Liver (2)	-	-	-	-	-	-	2+	-	-	-	-	-	-	-	-	-
Kidney (2)	2+	-	-	3+	-	-	-	-	-	-	-	1+s	-	-	-	2+
Testis (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Connective tissues																
Lung (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Breast (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prostate (2)	1+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colon (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stomach (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pancreas (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Uterus (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ovary (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liver (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kidney (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^a Data based on the indirect immunoperoxidase method.

^a All tissues were stained by the ABC immunoperoxidase method except stomach, liver, and kidney, which were stained by the indirect immunoperoxidase method.^b The number in parentheses indicates the number of different specimens tested.^c Histocytes in the red pulp were predominantly stained.^d A few luminal cells were stained.^e (1/2), one of the two specimens was positive.^f Leydig cells were stained.^g Seminiferous tubules were stained.

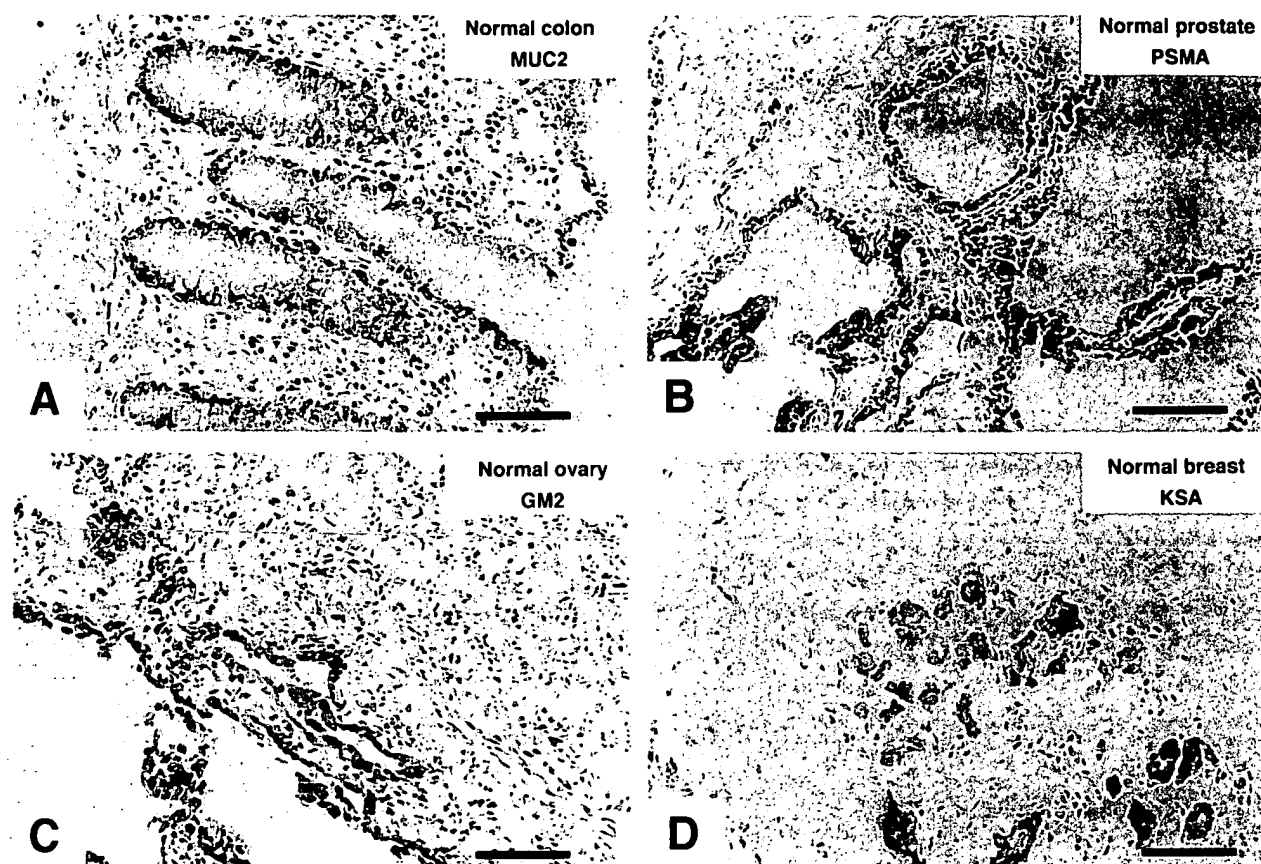


Fig. 2 Expression of the tumor-associated antigens on normal tissues. Luminal cells of normal epithelia were stained in colon with mAb LDQ10 against MUC2 (A), in prostate with mAb Cyt351 against PSMA (B), in ovary with mAb 696 against GM2 (C), and in breast with mAb GA733-2 against KSA (D). Note the heterogeneous expression of PSMA on prostate epithelia. Scale bar, 100 μ g.

PSMA were strongly expressed in 8–10 of 11 primary cancers. The frequently strikingly positive imaging of prostate cancers with sTn mAbs B72.3 and CC49 (22) and the immunohistological results in a recently completed study with anti-PSMA mAb CYT351 demonstrating strongly positive staining in seven of eight lymph node metastases (16, 17) have suggested that these two antigens are widely expressed on prostate cancers. Although there are no previous studies demonstrating the expression of GM2, KSA, TF, Tn, or MUC2 on prostate cancers, the surprising frequency and intensity of their staining on most of the primary and metastatic prostate carcinomas that we tested leave little doubt about their presence in prostate cancers and their suitability as potential targets for immune attack.

The widespread expression of most of these antigens on normal tissues is, at first look, disturbing. GM2 and KSA are expressed on the epithelial surfaces of nearly all tissues tested, whereas PSMA, hCG β , TF, Tn, sTn, and MUC2 showed weak to moderate staining on epithelial cells of between one and five organs. The only other expression of these antigens on normal tissues was moderate expression of GM2 in brain gray matter and expression of sTn, hCG β , and KSA in the testis. There are now sufficient data from clinical trials with vaccine-induced antibody responses against GM2, GD2, MUC1, hCG β , Globo H, TF, and sTn antigens (23–30) and passive administration of

mAbs against GD2, GD3, KSA, Globo H, and sTn (31–35) to draw conclusions about the consequences of antigen distribution on normal tissues. GM2, GD2, and GD3 exposure on cells in the brain (both GD2 and GD3 are more abundantly expressed than GM2), and GM2, sTn, MUC1, hCG β , and Globo H antigen expression in cells at secretory borders of epithelial tissues induced neither immunological tolerance nor autoimmunity once antibodies were present, suggesting that they are sequestered from the immune system. Our experience in patients with prostate cancer supports this suggestion. No normal organ toxicity was seen in prostate cancer patients producing antibodies against MUC1 or Globo H after immunization with conjugate vaccines,⁴ nor were normal epithelial cells detectably affected by treatment with ¹³¹I-labeled mAb against sTn (22). Treatment with mAbs against GD2 and GD3 has not induced central nervous system toxicity in children or adults (31–33). On the other hand, treatment with mAbs against some antigens present in locations other than brain and secretory epithelia have resulted in toxicity: high doses of some mAbs against GD2 (which is also expressed in some peripheral nerves) has resulted in

⁴ S. Slovin, H. Scher, and P. Livingston, unpublished observations.

Table 4 Correlation between expression of tumor-associated antigens on nine metastatic prostate cancers

Metastatic site	GM2 696	TF 49H.8	Tn 1E3	sTn B72.3	MUC1 HMFG-2	MUC2 LDQ10	hCGB FB12	PSMA Cyt351	KSA GA733-2
	95%	90%	85%	90%	60%	80%	—	—	60%
Bone	3+	3+	3-4+	3-4+	2-4+	2+	—	80%	2-3+
	90%	40%	80%	80%	5%	80%	—	3-4+	95%
Lung	4+	1+	2-3+	3-4+	3+	4+	80%	—	4+
	90%	70%	70%	30%	90%	90%	4+	90%	95%
Lymph node	4+	4+	2-3+	2-3+	3-4+	4+	30%	4+	4+
	60%	—	20%	—	—	40%	1+	90%	95%
Lymph node	2-3+	—	2+	—	—	2+	—	4+	3-4+
	60%	40%	20%	5%	10%	90%	—	95%	95%
Lymph node	2-4+	1+	2+	2+	2+	3+	—	4+	4+
	—	40%	20%	—	80%	95%	—	95%	90%
Lymph node	—	2+	2+	—	3+	4+	—	4+	3+
	90%	40%	—	—	70%	90%	—	95%	95%
Liver	2+	2+	—	—	3+	4+	—	4+	4+
	95%	30%	20%	90%	70%	90%	—	5%	95%
Liver	3+	2+	2+	4+	4+	4+	—	4+	4+
	90%	—	50%	—	—	90%	—	90%	95%
Brain	3+	—	2+	—	—	3+	—	4+	4+

peripheral neuropathies (36), and treatment with a mAb against Le^x (expressed at secretory borders but also on polymorphonuclear leukocytes) has resulted in striking, short-lived neutropenia (37, 38). Against this background, nine antigens (GM2, KSA, TF, Tn, sTn, hCGB, PSMA, MUC1, and MUC2) all appear to be excellent targets for immunotherapy of prostate cancer with vaccines or mAbs.

These results provide the basis for using combinations of antibodies or for polyvalent vaccines for immunotherapy of prostate cancer. We have screened for the expression of 30 different potential prostate cancer cell surface antigens (including 18 here and 12 additional antigens in our previous studies; Refs. 1 and 2) to identify the 9 most widely expressed antigens. However, none of the nine were strongly expressed on every prostate cancer cell, suggesting the need for a polyvalent vaccine or mixture of mAbs for immunotherapy. For instance, the four cases of metastatic prostate cancer with weak or moderate GM2 expression in our study showed strong expression of PSMA and KSA (see Table 4). All metastatic lesions showed strong expression of at least two of these eight antigens in at least 80% of tumor cells and strong expression of at least one additional antigen in at least 60% of cells. On the basis of these results, we have initiated a program aimed at augmenting the immune response against each of these nine antigens with a polyvalent prostate cancer vaccine.

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